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(PCT Rule 72.2)

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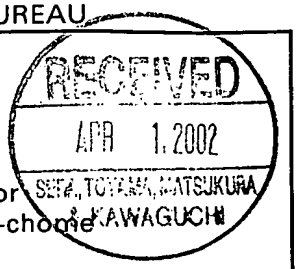

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Date of mailing (day/month/year) 19 March 2002 (19.03.02)	
Applicant's or agent's file reference B691SMOP1072	**IMPORTANT NOTIFICATION**
International application No. PCT/JP00/06913	**International filing date** (day/month/year) 04 October 2000 (04.10.00)
Applicant AJINOMOTO CO., INC. et al	

1. Transmittal of the translation to the applicant.

The International Bureau transmits herewith a copy of the English translation made by the International Bureau of the international preliminary examination report established by the International Preliminary Examining Authority.

2. Transmittal of the copy of the translation to the elected Offices.

The International Bureau notifies the applicant that copies of that translation have been transmitted to the following elected Offices requiring such translation:

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3. Reminder regarding translation into (one of) the official language(s) of the elected Office(s).

The applicant is reminded that, where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report.

It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned (Rule 74.1). See Volume II of the PCT Applicant's Guide for further details.

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Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference B691SMOP1072	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/JP00/06913	International filing date (*day/month/year*) 04 October 2000 (04.10.00)	Priority date (*day/month/year*) 04 October 1999 (04.10.99)
International Patent Classification (IPC) or national classification and IPC C12N 15/60, 15/54, 15/53, 15/31, 15/56, 9/88, 9/12, 9/04, C07K 14/34, C12N 9/26, C12P 13/04		
Applicant AJINOMOTO CO., INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10 April 2001 (10.04.01)	28 September 2001 (28.09.2001)
Name and mailing address of the IPEA/JP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP00/06913

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:

pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:

pages _____, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:

pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:

pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-49	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-49	NO
Industrial applicability (IA)	Claims	1-49	YES
	Claims		NO

2. Citations and explanations

Document 1: JP, 7-63383, B2 (12.07.95)

Document 2: JP, 4-4887, A (09.01.92)

Document 3: K. Takai et al., "ppc, the gene for phospho-enolpyruvate carboxylase from an extremely thermophilic bacterium, Rhodothermus obamensis: Cloning, sequencing and over-expression in Escherichia coli", Microbiology (1988), Vol. 144, No. 5, pp. 1423-1434

Document 4: JP, 5-56782, A (09.03.93)

Document 5: WO, 92/18635, A1 (29.10.92)

Document 6: D. Wereecke et al., "Cloning and sequence analysis of the gene encoding isocitrate lyase from Rhodococcus fascians", Gene (1994), Vol. 145, No. 1, pp. 109-114

Document 7: W. Jager et al., "A Corynebacterium glutamicum gene encoding a two-domain protein similar to biotin carboxylases and biotin-carboxyl-carrier proteins", Arch. Microbiol. (1996), Vol. 166, No. 2, pp. 977-984

Document 8: S. Donadio et al., "Erythromycin production in Saccharopolyspora erythrae does not require a functional propionyl-CoA carbocyclase", Mol. Microbiol. (1996), Vol. 19, pp. 977-984

Document 9: WO, 94/08016, A1 (14.04.94)

Document 10: WO, 96/32484, A2 (17.10.96)

Document 11: E. Kimura et al., "Molecular cloning of a novel gene, dtsR, which rescues the detergent sensitivity of a mutant derived from Brevibacterium lactofermentum", Biosci. Biotechnol Biochem. (1996), Vol. 60, pp. 1565-1570

Document 12: WO, 95/23224, A1 (31.08.95)

Document 13: JP, 10-234371, A (08.09.98)

Document 14: JP, 7-121227, B2 (25.12.95)

Document 15: A. M. Alves et al., "Characterization and phylogeny of the pfp gene of Amycolatopsis methanolica encoding PPi-dependent phospho-fructokinase", J. Bacteriol. (1996), Vol. 178, pp. 149-155

Document 16: A. M. C. R. Alves et al., "Identification of ATP-dependent phosphofructokinase as a regulatory step in the glycolytic pathway of the actinomycete Streptomyces coelicolor A3 (2)", Appl. Environ. Microbiol. (1997), Vol. 63, pp. 951-956

Document 17: JP, 8-196280, A (06.08.96)

Document 18: JP, 5-244958, A (24.09.93)

Document 19: W. Kronemyer et al., "Structure of the gluABCD cluster encoding the glutamate uptake system of Corynebacterium glutamicum, "J. Bacteriol. (1995), Vol. 177, pp. 1152-1158

Document 20: C. Rollin et al., "^{13}C-NMR studies of Corynebacterium melassecola metabolic pathways", Eur. J. Biochem. (1995), Vol. 227, No. 1-2, pp. 488-493

Document 21: S. Hein et al., "Biochemical and molecular characterization of the Alcaligenes eutrophus pyruvate dehydrogenase complex and

identification of a new type of dihydro-lipoamide dehydrogenase", J. Bacteriol. (1994), Vol. 176, pp. 4394-4408

Document 22: P. E. Stephens et al., "The pyruvate dehydrogenase complex of Escherichia coli K12. Nucleotide sequence encoding the pyruvate dehydrogenase component", Eur. J. Biochem. (1983), Vol. 133, pp. 152-162

Document 23: WO, 99/18228, A2 (15.04.99)

Document 24: JP, 10-165180, A (23.06.98)

Document 25: JP, 2-291276, A (18.04.90)

Document 26: JP, 11-196887, A (27.07.99)

Document 27: JP, 8-66189, A (12.03.96)

Document 28: M. P. Ruklish et al., "The functioning of the tricarboxylic acid cycle in Brevibacterium flavum and Micrococcus glutamicus", Mikrobiologiya (1987), Vol. 56, No. 5, pp. 759-763

Document 29: J. M. Mengaud et al., "The major iron-containing protein of Legionella pneuphilia is an aconitase homologous with the human iron-responsive element-binding protein", J. Bacteriol. (1993), Vol. 175, pp. 5666-5676

Document 30: C. Prodromou et al., "The aconitase of Escherichia coli. Nucleotide sequence of the aconitase gene and amino acid sequence similarity with the mitochondrial isopropyl-malate isomerases", Eur. J. Biochem (1992), Vol. 204, pp. 588-609

Document 31: B. J. Eikmanns et al., "Cloning and sequence analysis, expression and inactivation of the Corynebacterium glutamicum icd gene encoding isocitrate dehydrogenase and biochemical characterization of the enzyme", J. Bacteriol. (1995), Vol. 177, pp. 774-782

Document 32: A. Ishii et al., "Genes encoding two isocitrate dehydrogenase isoenzymes of a psychrophilic bacterium, Vibrio sp. strain ABE-1", J. Bacteriol. (1993), Vol. 175, pp. 6873-6880

Document 33: B. J. Eikmanns et al., "Corynebacterium glutamicum lpd gene, complete CDS", Genbank (1 February 1999), Acc. No. Y16642

Document 34: WO, 97/48790, A1 (24.12.97)

Document 35: WO, 95/34672, A1 (21.12.95)

Document 36: E. R. Boermann et al., "Molecular analysis of Corynebacterium glutamicum gdh gene encoding glutamate dehydrogenase", Mol. Microbiol. (1992), Vol. 6, pp. 317-326

Document 37: JP, 6-502548, A (24.03.94)

Document 38: B. J. Eikmanns et al., "Nucleotide sequence, expression and transriptional analysis of the Corynebacterium glutamicum gltA gene encoding citrate synthase", Microbiology (1994), Vol. 140, pp. 1817-1828

Document 39: M. A. Pardo et al., "Nodulating ability of Rhizobium tropici is conditioned by a plasmid-encoded citrate synthase", Mol. Microbiol. (1994), Vol. 11, pp. 315-321

Claim 49

Documents 1 and 2 disclose methods for amino acid fermentation using the thermoduric bacterium Corynebacterium thermoaminogenes.

The disclosures in Documents 1 and 2 differ from the inventions set forth in Claims 1, 17 and 18 in the present application in that the former do not mention an isocitrate lyase of the specified amino acid sequence from Corynebacterium thermoaminogenes which contributes to amino acid synthesis, or a nucleic acid which encodes the

same.

However, it was known before the filing date of the present application that enzymes for industrial use are preferably heat-resistant enzymes, and cloning of heat-resistant enzymes from a thermoduric bacterium was a known problem; and Document 3 discloses a means for solving this problem by constructing a primer based on the nucleic acid sequence of the gene for a desired enzyme which contributes to amino acid synthesis in another, closely related, species, cloning the desired heat-resistant enzyme and determining the sequence of the gene coding the desired enzyme.

Documents 4-6 disclose amino acid sequences of bacterial isocitrate lyase from closely related species and sequences of the nucleic acid coding the same, and since construction of a primer and cloning do not entail any unexpected special difficulty for a person skilled in the art, the inventions set forth in Claims 1, 17 and 18 could be deduced easily by a person skilled in the art from Documents 1-6.

Production of transformant microorganisms using a cloned gene, and amino acid fermentation, were routine practices before the filing date of the present application; therefore, the same applies to Claim 49.

The fact that the resulting enzyme is heat-resistant is obvious given the nature of the microorganism from which it comes and cannot, therefore, be regarded as surprising.

Similarly, Documents 7-10 disclose amino acid sequences for acyl-CoA carboxylases and sequences of nucleic acid coding the same; Documents 11-13 disclose amino acid sequences for Dtsr and sequences of nucleic acid coding the same; Documents 14-16 disclose amino acid sequences for phosphofructokinase and sequences of nucleic

acid coding the same; Documents 17 and 18 disclose amino acid sequences for proteins which are able to bestow the ability to utilize sucrose, and sequences of nucleic acid coding the same; Claim 19 discloses amino acid sequences of proteins which have a function contributing to glutamic acid uptake, and sequences of nucleic acid coding the same; Documents 20-22 disclose amino acid sequences for pyruvate dehydrogenase and sequences of nucleic acid coding the same; Document 23 discloses an amino acid sequence for pyruvate carboxylase and the sequence of nucleic acid coding the same; Documents 24-27 disclose amino acid sequences for phosphoenolpyruvate carboxylase and sequences of nucleic acid coding the same; Documents 28-30 disclose amino acid sequences for aconitase and sequences of nucleic acid coding the same; Documents 31 and 32 disclose amino acid sequences for isocitrate dehydrogenase and sequences of nucleic acid coding the same; Document 33 discloses an amino acid sequence for dihydrolipoamide dehydrogenase and the sequence of nucleic acid coding the same; Documents 34-35 disclose amino acid sequences for 2-oxoglutarate dehydrogenase and sequences of nucleic acid coding the same; Documents 36 and 37 disclose amino acid sequences for glutamate dehydrogenase and sequences of nucleic acids coding the same; and Documents 38 and 39 disclose amino acid sequences for citrate synthase and sequences of nucleic acid encoding the same.

Therefore, the inventions relating to acyl-CoA carboxylases set forth in Claims 2, 19 and 20, the inventions relating to Dtsr set forth in Claims 3, 4 and 21-24, the inventions relating to phosphofructokinase set forth in Claims 5, 25 and 26, the inventions relating to proteins capable of bestowing the ability to utilize sucrose set forth in Claims 6, 27 and 28, the inventions relating to proteins having a function contributing to

glutamate uptake set forth in Claims 7, 29 and 30, the inventions relating to pyruvate dehydrogenase set forth in Claims 8, 31 and 32, the inventions relating to pyruvate carboxylase set forth in Claims 9, 33 and 34, the inventions relating to phosphoenolpyruvate carboxylase set forth in Claims 10, 35 and 36, the inventions relating to aconitase set forth in Claims 11, 37 and 38, the inventions relating to isocitrate dehydrogenase set forth in Claims 12, 39 and 40, the inventions relating to dihydrolipoamide dehydrogenase set forth in Claims 13, 41 and 42, the inventions relating to 2-oxoglutarate dehydrogenase set forth in Claims 14, 43 and 44, the inventions relating to glutamate dehydrogenase set forth in Claims 15, 45 and 46 and the inventions relating to citrate synthase set forth in Claims 16, 47 and 48 could be conceived easily from the aforementioned documents.